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49. (New) The method of claim 48, wherein detecting is accomplished by measuring intracellular calcium levels in the host cell.--

add E3

REMARKS

By this Second Supplemental Preliminary Amendment, the specification and claim 16 are amended, claims 19, 25, and 26 are cancelled, and new claims 38-49 are added. Currently, claims 16 and 38-49 are pending in this application.

The specification is amended to replace the Sequence Listing with a corrected version, to include Sequence Identifiers in accordance with the new Sequence Listing, to conform it to current PTO practice regarding explicit recitation of nucleic acid and amino acid sequences, and to include reference to the deposit information for clone Lyme21-9. As discussed in detail below, no new matter is added by the amendments to the specification.

Claim 16 is amended to conform it to current PTO practice regarding explicit recitation of nucleic acid and amino acid sequences. The amendment to claim 16 does not change the scope or content of the claim. New claims 38-49 are added to specifically recite embodiments of the presently claimed invention, and to recite a method of using the receptor of the invention. Support for new claims 38-44 comes from the specification, as originally filed, at pages 2-3, page 7, page 19, and Figure 1, for example. Support for new claims 45-49 comes from the specification, as originally filed, at pages 5-6, for example. Accordingly, no new matter is added by the amendment of claim 16, and new claims 38-49.

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Figure 3 depicts the alignment of the complete amino acid sequences for eight human chemotactic receptors together with the amino acid sequence deduced from the presently cloned cDNA (Lyme21-9) showing the high degree of similarity (shaded areas), not least within the transmembrane regions. The homology presentation was done with the SeqVu (version 1) mode in the GCG program. The scaling system used is described by Riek et al. (1995).

Figure 4 depicts a dendrogram (the horizontal distances to the branching points corresponding to the relative degree of sequence identities) based on the eight human chemoattractant receptors listed in Fig. 3 as well as the Duffy antigen, together with the currently cloned Lyme21-9, showing similarities in the evolutionary pattern. For comparison, the dendrogram also includes a selection of "unrelated" human receptors belonging to the rhodopsin-type family: the amine receptors histamine H1, muscarinic M1, dopamine D_2, adrenergic β_2, and the peptide receptors neuromedin K, substance P, and substance K. Full amino acid sequences were used in the multiple alignments and the dendrogram and they were performed with the PileUp software in the GCG program.

Figures 5A and 5B depict ~~Figure 5 depicts~~ fluorescence photomicrographs with examples of FISH mapping of the gene corresponding to CMKRL1. Fig. 5A shows fluorescent signals on one human chromosome. Fig. 5B shows

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Various (protein) concentrations of membranes were incubated with 2 nM tritiated LTB_4 for 60 min at 4°C, with or without 1 μM unlabeled LTB_4. Results are mean values of triplicate wells.

Figure 10 shows the action of 4 different concentrations of LTB_4 (figures given are final concentrations in the test well) on intracellular calcium levels in CHO cells stably transfected to express CMKRL1 and preloaded with Fura-2 for monitoring of intracellular calcium concentration (fluorescence ratios). The lower curve illustrates the effect of the highest LTB_4 concentration tested on sham-transfected control cells. Values are means ± SEM (n).

Figure 11 shows the effect of 1000 nM (final concentration in test well) of LTB_4 on intracellular calcium levels in CHO cells transfected to express CMKRL1 (and preloaded with Fura-2 for monitoring of calcium fluorescence ratios). Effects of LTB_4 are compared in calcium (Ca) containing and calcium-free cell media. Values are means ± SEM (n).

~~Figure 12 reports~~ Figures 12A and 12B report the results of fluorescence immunocytochemistry of a monoclonal antibody (mAb) raised against a synthetic peptide corresponding to the first 15 amino acid residues in the extracellular tail of CMKRL1 showing (a) finely-granular fluorescence in the periphery of CHO cells stably expressing CMKRL1, and (b) absence of fluorescence in sham-transfected control cells. Magnification: 500x.

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